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Extraction of Phytochemicals and Secondary Metabolite from *Euphorbia hirta*

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ABSTRACT

Plant Euphorbia *hirta* belongs to the family Euphorbiaceae is well known for its medicinal properties and widely used worldwide. Leaves of Euphorbia *hirta* investigated for its Ash value, extractive value and fluorescence analysis. Further, the present study aimed to identify the various phytochemical constituents present in the two different extracts by using standard methods of phytochemical screening. Powdered plant material contained 10.29% Ash value, 9.89 % w/w water soluble extract, 13.25% w/w methanol soluble extract, and fluorescence to some treatments. The chemical constituents of the methanolic leaf extracts of the plant were relatively similar in the presence of proteins as primary metabolites and alkaloids, flavonoids, terpenoids, tannin, steroid, saponin, carbohydrate as secondary plant metabolites. In contrast, aqueous extract appeared to possess the constituents such as carbohydrates, proteins, tannin, steroid, saponin and flavonoids with a negative result to alkaloids. In conclusion, the reported results support the leaf extracts of Euphorbia contain several phytochemicals which have therapeutic medicinal uses.

Key Words: Euphorbia hirta, phytochemical screening, metabolites

INTRODUCTION

Several plants have been widely used in traditional medicine all over the world, to treat several diseases such as skin ulcers and warts, as well as cancer tumours and intestinal parasites [1]. Euphorbia species are one of them [2]. Throughout ancient history Euphorbia have been used in folk medicine to treat various medical conditions. Medicinal plants have biologically compounds which are used for treating various human diseases and also play an important role in curing. Phytochemicals which are present in plants have two categories i.e., primary and secondary metabolites. Primary metabolites involve chlorophyll, proteins sugar and amino acids whereas secondary contain terpenoids and alkaloids. Because of the presence of these secondary metabolites medicinal plants show different activities like antifungal, antibacterial and anti-inflammation. Primary metabolic processes such as building and maintaining plant cell are because of the primary metabolites [3]. Plants make many chemical compounds for biological functions, including defence against insects, fungi and herbivorous mammals. Since thousand years the use of plant and plant extracts for medicinal purpose are observed. The oldest remedies known to mankind are herbal medicines. In higher plants flower colour and fruits of higher plants are imparted by flavonoids, one of the secondary metabolites. They have multiple biological effect including antioxidant, free radical scavenging

abilities, anti-inflammatory, anti-carcinogenic etc. Earlier study [4] suggested about their contribution to physiological functions such as seed maturation and dormancy. Presence of flavonoids has been reported from many plant species like *Agave Americana* [5], *Arachis hypogea* [6], *Lysium barbarum* [7] and *T. terrestris* [8].

Medicinal plants serve as an important source of bioactive molecules for novel therapeutic agents [9]. Now days, the herbal therapeutic agent used against many diseases. Isolated bioactive molecules are utilized in the synthesis of new drugs. Hence, Extraction plays an important role in the separation of phytochemical processing for the discovery and isolation of bioactive constituents from plant materials. India is known worldwide for its Ayurveda treatment. *Euphorbia hirta* is often used traditionally for various disorders. Earlier report suggested that whole plant contain anti-cancerous [10], anti-bacterial[11], anti-fungal [12], anti-viral [13], anti inflammatory [14] and anti-microbial [15] activities. It has also been reported that plant contain alkanes, triterpenes, phytosterols, tannins, polyphenols, and flavonoids. This describes the medicinal properties, chemical constituents and other important aspects of *Euphorbia hirta*.

The present study is based on photochemical screening of Euphorbia *hirta* extraction of bioactive and their antimicrobial properties against certain microflora.

MATERAL AND METHOD

Collection of plant material: Aerial Part (leafs, stem, seeds) of Euphorbia *hirta* were collected from the field.

Preparation of plant extract: leaves of Euphorbia *hirta* were washed with clean water, shade dried, crushed and powered with electrical grinder and then the dried powdered sample. After that leaves were stored in air tight containers at room temperature for further analysis. 30gm of the powder was extracted successively with 150ml of DDW and Methanol using water bath for 48 hrs., after which the solvent was collected in petri dishes and left to dry and then the crude extracts were stored at 4°C in airtight petri plates till the analysis was performed.

ISOLATION, IDENTIFICATION OF SECONDARY METABOLITES

Leaves of (Euphorbia *hirta*) were air dried and powdered, separately. Each of these extracted separately with 80% methanol on water bath [16] for 24 h. The methanol soluble fractions were filtered, concentrated *in vacuo* and aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr I), diethyl ether (Fr II) and ethyl acetate (Fr III) separately. Each step was repeated thrice for complete extraction, fraction I was discarded in each case because it contained fatty substance, whereas fraction II and fraction III were concentrated and used for determining flavonoids.

Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid ($10mLg^{-1}$ plant material for 2 h), filtered and filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled separately, neutralized by distilled water with repeated washings and concentrated *in vacuo.* Both fraction II and III were taken up in small volume of ethanol (2-5mL) before chromatographic examination.

Thin Layer Chromatography (TLC)

The freshly prepared Silica gel plates were air dried at room temperature; thereafter these were kept at 100 0 C for 30 minutes to activate and then cooled at room temperature and were used for analysis.

Each of the extract was co- chromatographed with authentic flavonoid as a marker (quercetin, luteolin, kaempferol and rutin). These plates were developed in an air tight chromatographic chamber saturated with solvent mixture (Benzene: Acetic Acid: Water: 125:72:3; [17]Wong & Francis, 1968). The developed plates were air dried and visualized under UV light by exposure to ammonia fumes. The mouth of a 100 mL containing concentrated NH_4OH was held in contact with each spot for about 5-10 seconds and fluorescent spots corresponding to that of standard markers were marked. The developed plates were also sprayed with 5% $FeCl_3$, 0.1% alcoholic $AlCl_3$ and kept in I_2 chamber separately. The coloured spots thus developed were noted and the Rf value of each spot was calculated. Several others solvent systems such as n- butanol, acetic acid, water (4:1:5), tertiary butanol, acetic acid, water (3:1:1) were also tested but the solvent system containing benzene, acetic acid, water (125:72:3) gave better results.

PYTOCHEMICAL ANALYSIS

The phytochemical properties (Alkaloids, Sterols, Saponin, Flavonoids, Proteins, Carbohydrate) were determined with the help of methods [18,19,20].

RESULT AND DISCUSSION

Phytochemicals are the constituents which are present in plant parts like leaf, root stem. These have the capability to produce different physiological activities in human beings. Euphorbia leaves were analysed in present study. Secondary metabolites like alkaloids, sterols, saponin, flavonoids, proteins, carbohydrate were present in studied plant. Protein and carbohydrate were absent in methanol extract whereas alkaloids were absent in aqueous extract. On TLC plates flavonoids, steroid and alkaloids were found in fluorescent bluish green spot. These compound have specific defence activities which protect plants from herbivorous.

Table 1: Data Showing Ash Values, Extractive values of Leaves of Euphorbia *hirta*

S. No.	Physio-Chemical Analysis	Yield (% w/w)
1.	Total Ash	10.20
2.	Extractive values Water soluble extract Methanol soluble extract	 9.89 13.25

Table 2: Phytochemical analysis of *Euphorbia hirta*

S. No.	Phytochemical	Test	Aqueous	Methanol
1.	**Alkaloids**	Hager's test Wagner's test Mayer's test	- - -	+ + +
2.	**Sterols**	Salkowaski test	+	+
3.	**Saponins**	Foam test	+	+
4.	**Flavonoids**	Shinoda test Alkaline reagent test	+ +	+ +
5.	**Protein**	Million's test Ninhydrin test	+ +	- -

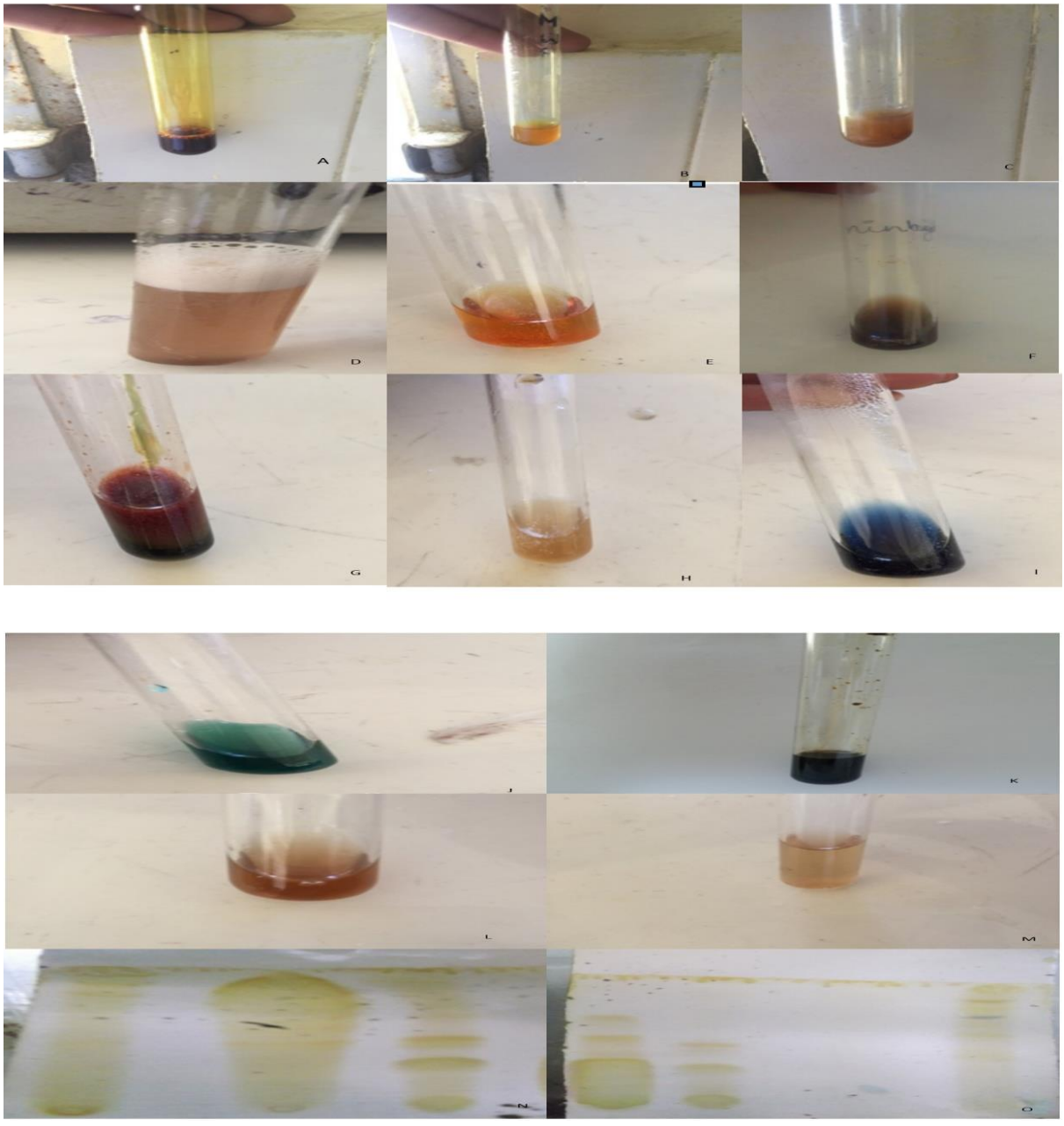
6.	**Carbohydrate**	Fehling's test	+	-
		Benedict's test	+	-
		Molish's test	+	-
		Iodine test	+	-

(+ Presence of compounds, - Absence of compounds)

Table:3 Quantitative analysis of phytochemicals in methanolic exyract of Ephorbia *hirta*

S. No.	Phytochemicals	Result (mg/gm)
1.	Flavonoids	68.45 ±4.12
2.	Saponins	52.85±2.15

Pictures showing the result of Phytochemical Analysis and Qualitative analysis of Flavonoids and sterols Ephorbia *hirta*



A. Wagner's Test (Alkaloids) B. Hager's Test (Alkaloids)
B. Mayer's Test (Alkaloids) D. Foam Test (Saponin)
E. Millon's Test (Protein) F. Ninhydrin Test (Protein)
G. Molisch's Test (Carbohydrate) H. Iodine Test (Carbohydrate)
I. Fehling's Test (Carbohydrate) J. Benedict Test (Carbohydrate)
K. Salkowskis Test (Sterol) L.Shinoda Test (Flavonoid)
M. Alkaline Reagent Test (Flavonoid)
N-O. Qualitative analysis of Flavonoids and sterols

CONCLUSION

Present study suggest that leaves of Euphorbia contain several phytochemicals which have therapeutic medicinal uses. Presence of flavonoids Euphorbia show the medicinal value like antioxidant, antiallergic and antimicrobial. Sufficient amount of flavonoids in plant tissue can be considered as an achievement for large the production of flavonoids being medicinally valuable.

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